

**DuPont Legal**

Barley Mill Plaza 11/1220
Wilmington, Delaware 19805
Facsimile: 302-992-5374

Facsimile Cover Sheet

To: Gabrielle Pegaiysky (sic!), Examiner

Company: US PTO, Art Unit 18XX?

Phone: 703-308-4201

Fax: 703-305-7401

From: Linda Axamethy Floyd, 33,692

Function: DuPont Legal

Phone: 302-892-8112

Fax: 302-992-5374

Date: 12 December 1997

Pages incl. this cover page: 3

Comments: PLEASE DELIVER PROMPTLY.

RE: Allowed claims for 08/687,852

Examiner:

Please confirm receipt of this fax as I failed to ask the spelling of your name or specific Art Unit. Please contact me should there remain any further issue to be resolved.


Linda Axamethy Floyd, USPTO Reg. No.: 33,692

*****Confidentiality Note*****

The documents accompanying this facsimile transmission contain information from DuPont Legal which is confidential and/or legally privileged. The information is intended only for the use of the individual or entity named on this transmission sheet. If you are not the intended recipient, you are hereby notified that any disclosure, copying, distribution or the taking of any action in reliance on the contents of this facsimiled information is strictly prohibited, and that any documents should be returned to DuPont legal immediately. In this regard, if you have received this facsimile in error, please notify us by telephone immediately so that we can arrange for the return of the original documents to us at no cost to you.

CR-9692-B

08/687,852

AMENDED CLAIMS

Please cancel Claims 20, 22, 23, 24, 26, 27 and 29.

14. The process of Claim 25 wherein the carbon substrate is selected from the group consisting of ethylene glycol, 1,2-propanediol, glycerol and 2,3-butanediol.

15. The process of Claim 14 wherein the carbon substrate is glycerol.

16. The process of Claim 15 wherein the glycerol is converted to 1,3-propanediol. \

17. The process of Claim 25 wherein the microbial host is selected from the group consisting of members of the genera *Eschericia*, *Bacillus*, *Klebsiella*, *Citrobacter*, *Saccharomyces*, *Clostridium* and *Pichia*.

18. The process of Claim 17 wherein the microbial host is selected from the group consisting of members of species *E. coli*, *Bacillus subtilis*, *Bacillus licheniformis* and *Pichia pastoris*.

19. The process of Claim 18 wherein the microbial host is *E. coli*.

21. The process of Claim 25 wherein (a) the transformed microbial host is recombinant *E. coli* DH5a containing a gene encoding an enzymatically active diol dehydratase enzyme, the gene comprising the DNA sequence of SEQ ID NO. 1; (b) the carbon substrate is glycerol; and (c) the product recovered in step(iii) is 1,3-propanediol.

25. A process for the bioconversion of a carbon substrate for diol dehydratase enzyme to the corresponding product comprising the steps of:

(i) transforming a microbial host with genes encoding an enzymatically active bacterial diol dehydratase enzyme, the genes derived from

(1) a cosmid, the cosmid comprising a DNA fragment of about 35 kb isolated from *Klebsiella pneumoniae* and contained within transformed *E. coli*

deposited with the American Type Culture Collection under accession number ATCC 69790; or from

(2) enzymatically active diol dehydratase genes isolated from the group consisting of members of the species *Klebsiella* sp., *Clostridia* sp., *Salmonella* sp. and *Citrobacter* sp, one subunit of the genes [and] ⁺ having at least a 95% identity to the nucleic acid sequence of SEQ ID NO:1;

(ii) contacting the transformed microbial host with the carbon substrate in a suitable medium; and

(iii) recovering the corresponding product from the suitable medium.

28. A process for the bioconversion of glycerol to 1,3-propanediol comprising the steps of:

(i) transforming a microbial host selected from the group consisting of the genera *Eschericia*, *Bacillus*, *Klebsiella*, *Citrobacter*, *Saccharomyces*, *Clostridium* and *Pichia* with genes encoding an enzymatically active bacterial diol dehydratase enzyme, the genes derived from a cosmid, the cosmid comprising: a DNA fragment of about 35 kb isolated from *Klebsiella pneumoniae*, the cosmid contained within transformed *E. coli* deposited with the American Type Culture Collection under accession number ATCC 69790; and

(ii) contacting the transformed microbial host with the carbon substrate in a suitable medium; and

(iii) recovering 1,3-propanediol from the suitable medium.

30. The process of Claims 21, 25 or 28 wherein the transformed microbial host further contains an alcohol dehydrogenase.

\\Wm\cgall\data\SHARED\Patent Documents\CR&D\CR-95xx-CR-96xx\CR-9692\b-amdcls.doc

* 312 amendment to
be filed Monday
15 Dec 1997.
Ltf